TIER 1 SCREENING SIDS DOSSIER ON THE HPV PHASE ..... CHEMICAL

CYCLOHEXANOL

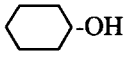
CAS No. 108-93-0

FINAL VERSION
December 30, 2005

CONTENTS

SIDS PROFILE

DATE: November 30, 2005

1.01 A.	**CAS No.**	108-93-0
1.01 C.	**CHEMICAL NAME**	CYCLOHEXANOL
1.01 D.	**CAS DESCRIPTOR**	Not applicable
1.01 G.	**FORMULA & STRUCTURE**	$C_6H_{12}0$ 
1.5	**QUANTITY**	1240 million pounds for 1998
1.7	**USE PATTERN**	Mainly used in the production of adipic acid and cyclohexylamine. Also used as an intermediate for pesticides, plasticizers, rubber chemicals, and pharmaceuticals; very limited use as a special process solvent.
1.9	**SOURCES AND LEVELS OF EXPOSURE**	Process leaks during manufacture of cyclohexanol, or conversion to other chemicals such as caprolactam, adipic acid and cyclohexylamine, could generate some vapor concentrations that may affect exposed personnel. There's also a low probability of skin contact, mainly for maintenance workers.
TEST PLAN JUSTIFICATION /ISSUES FOR DISCUSSION	SIDS testing conducted: A combined repeated-dose toxicity study and a reproductive/developmental toxicity screening study in rats (OECD 422), a hydrolysis study (OECD 111) in water, and an acute study for invertebrate toxicity (OECD 202). [Letter from IHF to EPA, 6-24-02]	

Tier 1

SIDS SUMMARY

DATE: December 30, 2005

CAS NO: 108-93-0		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL-CHEMICAL DATA								
2.1	Melting Point	Y	N	N			Y	N
2.2	Boiling Point	Y	N	N			Y	N
2.3	Density	Y	N	N			Y	N
2.4	Vapour Pressure	Y	N	N			Y	N
2.5	Partition Coefficient	Y	Y	N			Y	N
2.6	a. Water Solubility	Y					Y	N
	b. pH and pKa values							
2.7	Flash Point	Y						
2.8	Flammability	Y						
2.12	Oxidation: Reduction Potential	N						
2.13	Adsorption/Desorption to Sell	Y						
ENVIRONMENTAL FATE and PATHWAY								
3.1.1	Photodegradation	Y	N		Y	Y	Y	N
3.1.2	Stability in water	Y	Y	Y		N	Y	N*
3.3	Transport and Distribution	Y	N			Y	Y	N
3.5	Biodegradation	Y	Y			N	Y	N
ECOTOXICITY								
4.1	Acute toxicity to Fish[1]	Y	N	N			Y	N
4.2	Acute toxicity to Daphnia[1]	Y	Y	Y			Y	N*
4.3	Toxicity to Algae[1]	Y	Y	N			Y	N
TOXICITY								
5.1	Acute Toxicity							
5.1.1	Acute Oral	Y	N	N			Y	N
5.1.2	Acute Inhalation	Y	N	N			Y	N
5.1.3	Acute Dermal	Y	N	N			Y	N
5.4	Repeated Dose (General)	Y	Y	Y			Y	Y*
5.5	Genetic Toxicity *in vitro*	Y	N	N			Y	N
	. Gene mutation							
	. Chromosomal aberration							
5.6	Genetic Toxicity *in vivo*	Y	N	Y			Y	N
5.7	Reproduction Toxicity	Y	Y	Y	Y		Y	Y*
5.8	Developmental Toxicity/Teratogenicity	Y	Y	Y			Y	Y*

*The IHF Committee on Cyclohexanol accepted EPA's recommendation (Letter from the Industrial Health Foundation to EPA, June 24, 2002) to conduct a combined, repeated-dose, toxicity study and reproductive/developmental toxicity screening study (OECD 422) to satisfy HPV requirements. In addition to conducting those studies, the IHF Committee on Cyclohexanol
also conducted an OECD 111 study to measure the stability (hydrolysis) in water, and an OECD 202 study to better assess invertebrate toxicity.

1. GENERAL INFORMATION

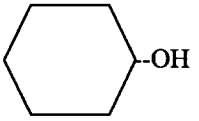
1.01 SUBSTANCE INFORMATION

A. CAS-Number 108-93-0

C. OECD Name Cyclohexanol

D. CAS Descriptor Not applicable

G. Structural Formula $C_6H_{11}OH$ (smiles code)



1.5 QUANTITY

Remarks: Cyclohexanol (and cyclohexanone) is primarily consumed either isolated or as a mixture in the production of adipic acid and caprolactam. According to company confidential data, approximately 1240 million pounds of cyclohexanol was produced in 1998. Less than 2% of this has typically been sold for use in other markets. The manufacturing process starts with either cyclohexane or phenol. (Stahl, W.F., Chemical Economics Handbook, SRI International – CEN Data Summary, Cyclohexanol and Cyclohexanone – United States, May 1998). According to that document, the merchant market for cyclohexanol was 27 million pounds in 1996 with over half (15 million pounds) used in the production of cyclohexylamine. More recently, however, the primary manufacturers of cyclohexylamine have apparently switched to aniline as the raw material of choice, making the market for cyclohexanol even less on a current basis.

Reference: Industrial Health Foundation, Pittsburgh, PA, June 18, 2001.

1.7 USE PATTERN

Remarks: Most of the cyclohexanol produced (~98%) is used in the production of adipic acid and caprolactam during the manufacture of nylon polymer. Other uses include:

a. Intermediate for agricultural chemicals (pesticides).
b. Intermediate for plasticizers
c. Intermediate for rubber chemicals.
d. Intermediate for pharmaceuticals.

Most of these uses involve further processing. Exposure to cyclohexanol in chemical processing is generally low because of the closed systems employed. Exposure of those using cyclohexanol as a chemical intermediate is expected to be similar to those found in manufacturing.

A limited amount of cyclohexanol is used as a solvent (primarily in special processes). The high melting and boiling points and low vapor pressure restrict its use as a general solvent. In these applications, appropriate handling guides (OSHA PEL, ACGIH TLV® or equivalent) have been established to assure safe handling. The low vapor pressure (essentially a solid at room temperature) helps in reducing the potential for human exposure by inhalation.

1.9 SOURCES OF EXPOSURE

Process leaks during manufacture of cyclohexanol or conversion to other chemicals such as caprolactam, adipic acid and cyclohexylamine would give rise to some vapor concentrations that may affect exposed personnel. There is also a low probability of skin contact, primarily for maintenance workers.

2. PHYSICAL-CHEMICAL DATA

2.1 MELTING POINT

Value:	24°C
Decomposition:	No Data
Sublimation:	No Data
Method:	No Data
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	
Reliability:	[4] Not assignable because limited study information was available.
Reference:	BASF/AG Sicherheitsdatenblatt (MSDS), Cyclohexanol (6/22/93), Ludwigshafen, Germany

2.2 BOILING POINT

Value:	161.1°C
Pressure:	at 1013 kPa
Decomposition:	No Data
Method:	No Data
GLP:	Yes [ ] No [ ] ? [X]
Remarks:	No additional data
Reliability:	[4] Not assignable because limited study information was available.

Reference: Budavari, S.(ed.), The Merck Index, 11th Ed.,Rahway, NJ: Merck & Co., Inc., Whitehouse Station, NJ, 1989, p.426.

2.3 DENSITY

Type: Bulk density [ ]; Density [ ]; Relative Density [x]

Value: 0.9624

Temperature: 20/4°C

Method: No Data

GLP: Yes [ ] No [ ] ? [X]

Remarks: No additional data

Reference: Lide, D.R.(ed.), CRC Handbook of Chemistry and Physics, 75th Ed., Boca Raton (FL), CRC Press Inc., 1994-1995, pp. 3-125.

2.4 VAPOR PRESSURE

Value: 1.33 hPa (1.0 mmHg)

Temperature: 20°C

Method: calculated [ ]; measured [ ]

GLP: Yes [ ] No [ ] ? [X]

Remarks: No additional data

Reliability: [4] Not assignable because limited study information was available.

Reference: BASG/AG Sicherheitsdatenblatt (MSDS), Cyclohexanol (6/22/93), Ludwigshafen, Germany.

2.5 PARTITION COEFFICIENT $\log_{10}P_{ow}$

$\log_{10}$Pow: 1.25

Temperature: 25°C

Method: calculated [ ]; measured [X] according to OECD Guideline 107 – "Partition Coefficient (n-octanol/water; Flask-Shaking method)

Result: Evaluation of isolated component:
Cyclohexanol log Pow=1.25
Cyclohexanone log Pow= 0.86

Remarks: Test conditions:

25 ml octanol and 25 ml distilled H_2O, stationary phase: Megabore-capillary (DB-17), thickness of film: 1.0 mm, diameter: 0.53 mm, length: 30 m, stove temperature: 60-160°C, detector temperature: 250°C, sampler temperature: 250°C, carrier gas: N_2, columns heat pressure: 1.5 bar (absolute), total gas flow: 165 ml/min, injection amount: 2.0 ml, instrument: HP 5890 with auto sampler, detector: flame ionization detector average from 3 measurements

Test Substance: test substance= Anolon™ mixture:
53.6% Cyclohexanol
42.0% Cyclohexanone
4.4% other

GLP: Yes [ ] No [ ] ? **[x]**

Reliability: (2) valid with restrictions
Discrepancy between documented test parameters and standard methods, but scientifically, acceptable

Reference: BASF AG Laboratory of Analytical Chemistry, Unpublished Data (J.Nr.101745/01), 7/12/1988.

2.6 WATER SOLUBILITY

Value: 3.6 wt%

Temperature: 20°C

Description:
[ ]Of very high solubility
[ ]Of high solubility
[ ]Soluble
[X]Slightly soluble
[ ]Of very low solubility
[ ]Not soluble

Method: No information

GLP: Yes **[ ]** No **[ ]** ? **[X]**

Remarks: No additional data

Reliability: **[4]** Not assignable because limited study information was available

Reference: Budavari, S. (ed.), The Merck Index, 11th Ed., Rahway, NJ;
Merck & Co., Inc., Whitehouse Station, NJ, 1989, p.426.

2.7 FLASH POINT: 68°C (SF Closed Cup)

2.8 AUTO FLAMMABILITY: 285°C (DIN 51794)

2.12 OXIDATION:REDUCTION POTENTIAL – No Data

2.13 ADSORPTION/DESORPTION TO SOIL

Method: Syracuse Research Corporation Model

Remarks: Cyclohexanol is slightly soluble in water with a value of 3.6 wt% at 20°C. If released to soil, it is expected to exhibit high-to-very-high mobility in soil. It may leach through soil to groundwater. It will not hydrolyze in moist soil, but it may be subject to volatilization from surface soil based upon estimated rates for its volatilization from water. It may be subject to biodegradation in soil based on results seen in laboratory aqueous screening tests.

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

3.1.1 PHOTODEGRADATION

Method:

Type: Air [X]; Water [ ]; Soil [ ]; Other [ ]

Rate constant: 17.48 E-12 (cm^3/molecules-sec)

Method: Calculated using AOPWIN v1.90 SAR Model

Remarks: Atmospheric photo-oxidation potential was estimated using the submodel AOPWIN (Meylan and Howard, 2000a). The estimation methods employed by AOPWIN are based on the SAR methods developed by Dr. Roger Atkinson et al. that rely on structural features of the subject chemical. The model calculates a second-order half-life with units of cm^3/molecules-sec. Photodegradation based on atmospheric photo-oxidation is based on the second order rate of reaction with hydroxyl radicals (HO), ($k2_{phot}$ with units of cm^3/molecules-sec). Default AOPWIN assumptions for calculation of first-order half-lives include an HO concentration of 1.5 E+6 molecules/cm^3 and 12 hours of daylight each day. Pseudo first-order half-lives ($t_{1/2}$) were then calculated as follows: $t_{1/2}=0.693/k2_{phot}$ x HO x 12-hr / 24-hr.

For cyclohexanol, the $k2_{phot}$ value was calculated to be 17.48 E-12 cm^3/molecules-sec and the resulting half-life was $t_{1/2}$ = 0.612 days or 14.7 hours.

Reliability: [2] Valid with Restrictions

Reference: Meylan, W. and P.H.Howard. 2000a. User's Guide for AOPWIN, Version 1.9, Syracuse Research Corporation, North Syracuse, NY, March, 2000.

3.1.2 STABILITY IN WATER

Method: Calculated [ ]Measured **[X]** according to OECD Guideline 111: Determination of Hydrolysis as a Function of pH.

- Type of Test: air [ ] water**[X]** soil[ ]
- GLP: Yes[X] No[ ] ?[ ]
- Year (performed): 2003
- Remarks:

Buffer solutions at pH 4.0, 7.0 and 9.0 were prepared, filtered through a 0.2mm membrane filter to ensure sterility before commencement of the tests, and then subjected to ultrasonification and degassing with nitrogen to minimize dissolved oxygen content. Sample solutions were prepared in staggered flasks at a nominal concentration of 0.95g/L in the three buffer solutions; solutions were shielded from light while maintained at the test temperature. For the actual testing, sample solutions at pH 4.0, 7.0 and 9.0 were maintained at 50.0 ±5°C for a period of 120 hours. Aliquots of the sample solutions were taken from the flasks at various times and the pH of each solution was recorded. The concentration of test material in the sample solution was determined by gas chromatography (GC). Duplicate aliquots of sample solution were diluted by a factor of 10 using methanol. Duplicate standard solutions of test material were prepared in methanol:relevant buffer solution (90:10 w/v) at a nominal concentration of 100 mg/L. Both the standard solutions and the sample solutions were analyzed by GC. The linearity of the detector response with respect to concentration was assessed over the nominal concentration range of 0 to 200 mg/L; this was satisfactory with a correlation coefficient of 1.0.

Results: The estimated half-life of cyclohexanol at 25°C is shown below:

pH	Estimated half-life
4	>1 year
7	>1 year
9	>1 year

Conclusion: Cyclohexanol is not expected to hydrolyze in water as evidenced by its projected half-life of greater than one year at a pH range of 4.0 to 9.0.

Data Quality (Klimisch Code): **[1]** Valid without restrictions

Reference: A.J. Evans, SafePharm Laboratories, Ltd. Cyclohexanol: Determination of Hydrolysis as a Function of pH. SPL Project Number 1592/006, 2003.

3.2 Transport and Distribution between Environmental Compartments Including Estimated Environmental Concentrations and Distribution Pathway

Method: Calculation according to Mackay, Level III, fugacity-based models obtained from Trent University's Modeling Center, Specific model: Equilibrium Concentration Model (EQC) Level 3 Model, Version 1.01.

Remarks: Default values were assumed for environmental compartment descriptions, dimensions, and properties, adjective and dispersive properties. Chemical specific parameters were: molecular weight (100.16 g/mol), Henry's Law Constant (4.44 E-6 atm-m3/mol), vapor pressure

(0.65 mm Hg), log Kow (1.23), air half-life (14.7 hr), water and soil half-lives (360 hr), sediment half-life (1440 hr), and equal loadings to air, water, and soil.

Results: Distribution was as follows:
Air (2.25%)
Water (50.2%)
Soil (47.5%)
Sediment (<0.1%)

Reliability: **[2]** valid without restriction

Reference: Meylan, W. and P.H. Howard, 2000a. User's Guide for AOPWIN, Version 1.9 Syracuse Research Corporation. North Syracuse, NY. March, 2000.

Mackay, D. et al. 1996a. The fate of new and existing chemicals: a five-stage process. *Environ. Toxicol. Chem.* 15(9): 1618-1626.

Mackay, D. et al. 1996b. Evaluating the environmental fate of a variety of types of chemicals using the EQC model. *Environ. Toxicol. Chem.* 15(9): 1627-1637.

3.5 BIODEGRADATION

Type: aerobic **[X]**; anaerobic **[ ]**

Inoculum: non-adapted

Concentration of the chemical: 398 mg/l related dissolved organic carbon (DOC)

Medium: activated sludge
Degradation: = 98% after 6 days

Kinetics: 11% after 3 hours
45% after 1 day
98% after 4 days

Method: OECD Guideline 302B. "Inherent biodegradability: Modified Zahn-Wellens Test"

Test substance: as prescribed by 1.1-1.4

Results: Concentration: 131 ml/l
DOC = 398 mg/l, AOX <1 mg/l
Elimination after 3 hours: 370 mg/l DOC
After 6 days: 24 mg/l

Test Conditions: Steam solution: DOC = 3060 mg/l
AOX <3mg/l, pH = 7.7 value = 300 ml, inoculumn = 150 mg/l

GLP: Yes **[ ]** No **[ ]** ? **[X]**

Reliability: [2] valid with restrictions

Reference: BASF AG, unpublished data, LGU 87-758, 2.2/6187, 10/8/1990

4. ECOTOXICOLOGICAL DATA

4.1 ACUTE TOXICITY TO FISH

A. Preferred Result

Type of test: static [ ]; semi-static [ ]; flow-through **[X]**; other

Species: Pimephales promelas (fathead minnow) from Environmental Research Laboratory, Diluth culture

Exposure period: 96 hours

Results: 96-hour LC50 = 704 mg/l (CL not relevant)

Analytical monitoring: Yes

Method: Test method of the USEPA Committee on Methods for Toxicity (1975). Approximately 25 fish, about 29 days old, were exposed for 96 hours to nominal cyclohexanol concentrations of 0, 133, 222, 369, 616 and 1026 mg/L; each concentration was run in duplicate. Analytically measured concentrations for each group (and its replicate) were: <0.7 (<0.7), 120 (124), 183 (185), 304 (310), 532 (533) and 942 (952) mg/L. During the exposure period, the average temperature of the test medium was 24.4°C±0.72°C (mean±ISD).

Test substance: Purity 99%

GLP: Yes [ ] No [ ] ? **[X]**

Remarks: At 96 hours, 100% mortality was observed at the highest dose level. No mortality was seen at other doses or in the control group at 96 hours. Affected fish lost equilibrium prior to death. Fish in the tank did not school after 30 hours of exposure. The 96-hr LC50 was calculated using the trimmed Spearman-Karber method on a PDP 11/70 computer.

Reliability: **[2]** valid with restrictions

Reference: Brooke, L.T., et al. Acute Toxicity of Organic Chemicals to Fathead Minnows, Vol. 1 Center for Lake Superior Environmental Studies, University of Wisconsin, 1982.

B. Supporting Data

The preceding study and 9 to 11 other freshwater fish studies have been conducted on cyclohexanol and are reported in USEPA's ECOTOX Report (November 27, 2000). All show the same low order of acute toxicity to freshwater fish.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. Preferred Result

Type of test: static [ ]; semi-static [X]; flow-through [ ]; other

Species: *Daphnia magna*

Exposure period: 48 hours

Results: EC_0 = 5.4 mg/L
EC_{50} = 17 mg/L (14 - 20 mg/L 95% CL)
EC_{100} = 56 mg/L (nominal)

Analytical monitoring: Yes [X] No [ ]

Method: OECD Guideline 202 – "Daphnia sp, Acute Immobilization Test and Reproduction Test; referenced as Method C.2 of Commission Directive 92/69/EEC.

Test substance: Cyclohexanol; purity> 99%

GLP: Yes [X] No [ ]

Remarks: Following a preliminary range-finding test, 20 daphnids (2 replicates of 10 animals) were exposed to an aqueous solution of cyclohexanol at concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L for 48 hours at a temperature of approximately 21°C under semi-static test conditions. The number of immobilized daphnias was recorded after 24 and 48 hours.

Reliability: [2] Valid with restrictions. Analysis of the freshly prepared test concentrations at 0 and 24 hours showed the measured test concentrations to range from 81% to 107% of the nominal values. However, analysis of the old or expired test preparations sampled at 24 and 48 hours showed a marked decline in the measured test concentrations ranging from 49% to 105% of nominal. These results were in line with the preliminary stability analyses indicating that the test solutions were unstable under the test conditions employed. Although efforts were made to minimize losses due to the suspected volatile nature of the test material, such as using completely-filled, ground-glass, staggered, conical flasks as exposure vessels, losses may also have occurred by this route.

Reference: Wetton, P.M. and J. McKenzie, SafePharm Laboratories. Acute Toxicity to Daphnia Magna. SPL Project No. 1592/005, 2003.

B. Supporting Data

Type of test: static [ ]; semi-static [ ]; flow-through [ ]; other [X] ?

Species: *Daphnia magna*

Exposure period: 48 hours

Results: EC_0 = 250 mg/L
EC_{50} >500 mg/L
EC_{100} >500 mg/L

Analytical monitoring: Yes [ ] No [X]

Method: Directive 84/449/EEC, C.2 "Acute Toxicity for Daphnia" (1998)

Test substance: Cyclohexanol (C6H11O), produced at BASF AG in Ludwigshafen (batch number: B7/11/87, commercial product) with:
Purity of >99%
Molecular weight: 100.16 g/mol
Color: Colorless
Water solubility: 40 g/L (20°C)
Homogeneity: homogeneous

GLP: Yes [ ] No [ ] ? [X]

Remarks: Test water has a pH of 7.9 a total hardness of 2.55 mmole/L, an alkalinity up top 4.3 of 0.85 mmole/L, a conductivity of 550-650 ms/cm, a test temperature of 292-294°K, and an oxygen content of >2 mg/L.

Reliability: [2] Valid with restrictions

Reference: BASF AG, Department of Ecology, Unpublished Data (1111/87), 1/15/1988.

4.3 ACUTE TOXICITY TO AQUATIC PLANTS e.g. Algae

Type of test: static [X] semi-static [ ] flow-through [ ] other [ ]

Species: *Scenedesmus subspicatus* (Algae)

Exposure period: 72 hours

End-point: Growth rate

Results: 72-hr EC50 = 29.2 mg/L
72-hr EC20 = 0.11 mg/L
96-hr EC20 = 0.22 mg/L
96-hr EC50 = 29 mg/L
96-hr EC90 = 470 mg/L

Analytical monitoring: Yes [ ] No [X]

Method: DIN 38412, Part 9, "Determination of inhibitory effection on cell multiplication" (1988)

Test substance: Cyclohexanol (C6H11O), produced at BASF AG in Ludwigshafen (batch number: B7/11/87, commercial product) with:
purity of >99%
molecular weight: 100.16 g/mol
color: colorless
water solubility: 40 g/L (20°C)
homogeneity: homogeneous

GLP: Yes [ ] No [X]

Remarks: The duration of the entire test was 96 hours. Inoculum density was 10,000 cells/ml, test temperature was 293°K, initial pH was 9.7 and pH range was 8 to 9.7; illumination: artificial light – permanent illumination, intensity of 120 E/m2a

Reliability: [2] valid with restrictions

Reference: BASF AG, Department of Ecology, unpublished data (1111/87), 1/22/1988.

5. TOXICITY

5.1.1 ACUTE ORAL TOXICITY

A. Preferred Result

Type of Test: LD_{50}

Species: Sprague-Dawley albino rats

Value: 1550 mg/kg (1390-1710 mg/kg CL)

Method: Consistent with OECD Test Guideline 401; single oral dose, undiluted; 2 to 3 rats/sex/dose; average weight at dosing 225 to 240 g; doses of 1000, 1260, 1580, 2000, 2510 and 3160 mg/kg were used

Test substance: cyclohexanol (>90% purity)

GLP: Yes [ ] No [ ] ? [X] (see remarks)

Remarks: Most deaths occurred within 24 hours, a few within 48 hours; no deaths occurred at 1000 or 1260 mg/kg; clinical signs included weight loss, increasing weakness, ocular discharge, salivation, collapse and death. Gross autopsy results showed hemorrhagic lungs, discoloured liver, and acute GI inflammation in decedents; no gross findings of toxicity were seen in survivors at 14 days. This study was conducted prior to, but was consistent with, US GLP Guidelines Published in 21CFR58, 1978, and effective June 20, 1979.

Reliability: [2] valid with restrictions

Reference: Younger Laboratories. Project No. Y-78-73, OTSO53388617 (April 28) (TSCATS/424698), 1978.

B. Supporting Data:

Type: LD50

Species: Carworth-Wistar Rats

Value: 2060 mg/kg

Method: Single oral dose, undiluted; 5 rats/dose

Test substance: Purity not known

GLP: Yes [ ] No [X] ? [ ]

Remarks: No additional information

Reliability: [2] valid with restrictions

Reference: H.F. Smyth et al. Am.Ind. Hyg. Assoc. J. 23: 95-107, 1962.

5.1.2 ACUTE INHALATION TOXICITY

Type: LC50

Species: Sprague-Dawley rats (M/F)

Value: >3.63 mg/L

Method: A dynamic inhalation exposure involving head and nose was used. The dose was nominally 7.5 mg/L but was analytically determined to be 3.63 mg/L by gas chromatography. Cyclohexanol was administered as an aerosol (particle size unknown) to 10 male and 10 female rats. Body weight at the start averaged 185g±15g and rats were weighed 7 and 14 day after dosing.

Test substance: cyclohexanol with a purity of 99.9%

GLP: Yes [ ] No [X] ? [ ]

Remarks: No animals died during the 14-day observation period. The only clinical sign was "unkempt fur" and it occurred only during the exposure. At 14 days post dosing, gross autopsies were unremarkable. Body weight gain was similar for control and test rats.

Reliability: [2] valid with restrictions

Reference: BASF AG, Department of Toxicology, Unpublished studies (78/791), 4/19/79.

5.1.3 ACUTE DERMAL TOXICITY

Type: LD50

Species: New Zealand albino rabbits

Value: >501 <794 mg/kg

Method: Cyclohexanol was applied undiluted to the skin of rabbits for 24 hours, 1 male or 1 female rabbit/dose, at 7 doses ranging from 316 to 5010 mg/kg. Body weights ranged from 1.9 to 2.6 kg.

Test substance: cyclohexanol (> 90% purity)

GLP: Yes [ ] No [X] ? [ ]

Remarks: All deaths occurred within 24 hours. Weakness, collapse, and death. Gross autopsy of descendents showed lung hyperaemia, liver and spleen discoloration, enlarged gall bladder, darkened kidneys and GI inflammation. Survivors at 14 days showed no remarkable findings at gross autopsy.

Reliability: [2] valid with restrictions

Reference: Younger Laboratories. Project No. Y-78-37, OTSO538617 (April 20), TSCATS/424698, 1978.

5.4 REPEATED DOSE TOXICITY

Type: An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in rats: Repeat dose component

Species/strain: Sprague-Dawley [Crl: CD®(SD) IGS BR] Male and Female rats (15/sex/exposure level)

Route of Administration: Inhalation

Exposure period: 16 weeks per males;
13 weeks per females

Frequency of treatment: 6 hours/day, 5 days/week

Post exposure observation period: 4 weeks (5 rats/sex/exposure level)

Exposure Levels: 0, 50, 150 and 450/400 ppm (v/v); after 10 weeks exposure, the 450 ppm level was reduced to 400 ppm due to slight mortality and the perceived additional stress of mating (females).

Control group: Yes (air alone)

Method: As part of a modified OECD Guideline 422 Study, a repeat-dose toxicity study was conducted. The only modifications to the original OECD 422

repeat dose protocol were an extension of the exposure period for both males (16 weeks) and females (13 weeks), and a 4-week recovery period to assess reversibility. Rats were exposed by whole-body inhalation to cyclohexanol vapor at design exposure levels of 0, 50, 150 and 450 ppm (v/v), respectively. Both male and female rats were exposed 6 hours/day, 5 days/week for either 13 weeks (females) or 16 weeks (males), after which 5 rats/sex/group were selected for a 4-week recovery period. Because of slight mortality seen at 450 ppm and concern about the effect of additional stress (males) of mating, the 450 ppm exposure level was lowered to 400 ppm after 10 weeks exposure (the beginning of the mating period; males from the repeat dose component were also used in the reproductive component of this study). Measurements on the animals included clinical signs, neurobehavioral observations, body weights and food consumption. After 5 weeks (5 animals/sex/group), 13 weeks (10 females/group), 18 weeks (10 males/group), and after the 4-week (5 rats/sex/group) recovery period, designated rats were selected for blood collection to evaluate hematology, clinical chemistry and urinalysis parameters. Complete necropsies were performed on all rats and specific organs and tissues were weighed and examined microscopically.

Results: The overall analytical mean (±SD) exposure levels for repeat-dose inhalation study of cyclohexanol were 50±9, 151±25, 449± 82 (1st 10 weeks) and 403±43 ppm (last 6 weeks) of cyclohexanol in air. Single males were found dead at 450 ppm on Days 37, 38 and 60 of the study. A single female rat was euthanized in extremis on Day 17. Although no cause of death could be determined, they are probably treatment-related since they all occurred at the high-exposure level. In the weekly detailed clinical observations, no compound related effects were seen. However, in observations conducted immediately post-exposure, adverse clinical signs such as decreased activity and prostration were seen in a few animals (both sexes) in the 450/400 ppm exposure group. No compound-related effects were seen relative to ophthalmoscopic evaluations, Functional Observational Battery (FOB), motor activity, body weight gain, food consumption, hematology endpoints, clinical chemistries, urinalysis, organ weights, or macroscopic and microscopic evaluations at any test level. Based on slight mortality and some adverse clinical signs seen immediately post-exposure in high level (450/400 ppm) exposure group only, the NOEL for the repeat dose component of this modified OECD 422 study was considered to be 150 ppm.

Conclusion: When rats were exposed repeatedly by inhalation for 6 hours/day, 5 days/week for 13 weeks (females) or 16 weeks (males), the only treatment-related effects observed were a slight increase in mortality and some adverse clinical signs (prostration and decreased activity) immediately post-exposure in a few rats in the 450 ppm exposure group. The NOEL for this repeat dose study of cyclohexanol in rats was considered 150 ppm.

Data Quality (Klimisch Code): **[1]** Valid without restrictions

Reference: Newton, P.E. (MPI Research, Inc.). An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in Rats with Cyclohexanol, Report No. 683-004, 2005.

5.5 GENETIC TOXICITY IN VITRO

A. Bacterial Test

(1) Type: Bacterial reverse mutation assay

System of testing: Standard plate method

Concentration: cyclohexanol concentrations ranged from 500 µg/plate to 10,000 µg/plate (without metabolic activation) or 15,000 µg/plate (with metabolic activation)

Method of Activation: With [ ]; Without [ ]; With and Without [X]; No data [ ]

Results: "Not mutagenic"

Test Substance: Purity unknown

Cytotoxicity Concentration: 7500 µg/plate, with and without metabolic activation

Precipitation Concentration: Not applicable

Genotoxic effects: Negative, with and without metabolic activation

Method: Four histidine-requiring strains of *Salmonella typhimurium* bacteria were used (TA 1535, TA 1537, TA 1538 and TA 98). Two replicates were used at each test substance concentration and all tests were performed in the presence and absence of a rat-liver homogenate (S.9). Approximately 10^8 bacteria were used in each place and all plates were incubated at 37°C for 48 hours. Both positive (ethanol) and negative controls (2 AA, MNNG, et al.) were used in these studies.

GLP: Yes [ ] No [X] ? [ ]

Reliability: [2] valid with restrictions

Reference: DuPont Company, unpublished studies, Haskell Laboratory Report No. 755-75, 1975.

(2) Type: Other Point Mutation Assays in Bacteria (Supporting Data)

Summary:

Three other *in vitro* studies using *Salmonella typhimurium* bacteria were conducted on cyclohexanol. In two assays (Frantz 1981: Rowe and McCollister 1982), there was no evidence of mutagenicity but details were limited. In a third study (Haworth 1983), cyclohexanol tested at 3300 µg/plate and 9100 µg/plate, with and without metabolic activation, produced results relative to mutagenicity potential.

References:

- ❖ Frantz, S.W., and J.E. Sinsheimer. Mutation Research 90: 67-78, 1981.
- ❖ S. Haworth et al: Salmonella Test Results for 250 chemicals. Environ. Mutagen. Suppl. 1: 3-142, 1983.
- ❖ Rowe, V.K. and S.B. McCollister. Patty's Ind. Hyg. Toxicology, 3rd ed. Pp. 4644-4649, 1982.

B. Non-Bacterial *In Vitro* Test

Type: cytogenetic assay (chromosome aberration)

System of testing: human leukocytes

Concentration: 0.01, 0.001 and 0.0001 moles/l cyclohexanol were tested.

Metabolic activation: With [ ]; Without [X]; With and Without [ ]; No data [ ]

Results: "Positive"

Cytotoxicity Concentration: Unknown

Precipitation Concentration: Unknown

Genotoxic effects: without metabolic activation, cyclohexanol was reported to induce achromatic regions, breaks and deletions in chromosomes.

Method: Human Leukocyte Assay described by Morhead (1960).

GLP: Yes [ ] No [X] ? [ ]

Test substance: No data

Remarks: limited technical details; non-validated protocol; non-GLP

Reliability: [4]

Reference: Morhead, P.S., et al. Exper. Cell. Res. 20: 613-616, 1960. Collins, J.P. Diabete 19 (4): 215-221, 1971 (CA77:1583u).

5.6 GENETIC TOXICITY *IN VIVO*

(A) Type: Micronucleus Assay

Species/strain: NMRI Mice

Sex: Female [ ]; Male [ ]; Male/Female [X]; No data [ ]

Route of Administration: oral gavage

Exposure period: 16, 24 and 48 hours for the high dose group; 24 hours for the lower doses

Doses: 500, 1000, and 1500 mg/kg bw

Results: "Negative"
Animals, receiving the positive and negative control treatments showed no signs of toxicity, but mice given cyclohexanol did have toxic signs. The frequency of erythocytes containing micronuclei was similar between negative controls and the 3 cyclohexanol dose groups (including all time points for the high-dose group).

Effect on mitotic index or P/N ratio: No information

Genotoxic effects: Not an *in vivo* mutagen

Method: According to Schmid, W.: The Micronucleus Test, In: Kilbey et al. (eds.), Handbook of Mutagencity Test Procedures, Amsterdam-New York, Elsevier, 1977.
The test substance was suspended in an aqueous 0.5% carboxymethyl cellulose (CMC) formulation. It was given to male and females in a volume of 10 ml/kg. The negative control received merely the carrier solution. The positive control for clastogenicity was 20 mg/kg bw of cyclophosphamide in distilled water using a volume of 10 ml/kg. The positive control for spindle poisoning effects was 0.15 mg/kg bw of vincristine in distilled water using a volume of 10 ml/kg. Five males and five females were used per dose. Animals were sacrificed at the times indicated and bone marrow from both femurs were prepared. After staining, 1000 polychromatic erythrocytes were evaluated per animal and examined for micronuclei. The normocytes with and without micronuclei occurring per 1000 polychromatic erythrocytes were also recorded.

GLP: Yes [X] No [ ] ? [ ]

Test substance: 98.8% pure cyclohexanol

Remarks: Under these experimental conditions, cyclohexanol has no chromosome-damaging (clastogenic) effects, nor does it lead to any impairment of chromosome distribution in mitosis.

Reliability: [1] valid without restrictions

Reference: BASF AG, Department of Toxicology, unpublished studies (89/843), 10/29/91.

(B) Type: Gene Mutation *In Vivo* (Supporting Data)

Summary: Cyclohexanol at 0.1 ml/100ml was given to *Drosophila melanogaster* (fruit flies) for 3 days as part of an SLRL Test. The results of this non-GLP test were negative, i.e., the frequency of recessive lethal mutations was not affected by treatment with cyclohexanol, even when followed by gamma and x-ray (1500R) irradiation.

Reference: R.I. Goncharova. Genetic Activity of Some Cyclohexane Derivatives. Genet. Tsito., pp. 137-142, 1970 (CA76:54780s).

5.7 TOXICITY TO REPRODUCTION

(A) Preferred Result

Type: An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in Rats: Reproductive Component.

Species/strain: Sprague-Dawley [Crl: CD®(SD)IGS BR] Rats (15/sex/exposure level)

Route of Administration: Inhalation

Exposure period: 10 weeks prior to mating and up to 6 more weeks during mating, gestation, and postpartum

Frequency of treatment:
Males: 6 hours/day, 5 days/week for 16 weeks
Females: 6 hours/day, 5 days/week for 10 weeks; 6 hours/day, 7 days/week for the next 6 weeks (during mating, gestation and postpartum)

Postexposure observation period: 5 rats/sex/group; 4 weeks

Exposure Levels: 0, 50, 150 and 450/400 ppm (v/v); after 10 weeks exposure the 450 ppm level was reduced to 400 ppm due to slight mortality and the perceived additional stress of mating.

Control Group: Yes (air alone)

Method: As part of a modified OECD Guideline 422 Study, a reproduction screening study was conducted. The only modifications to the original OECD 422 reproductive protocol, were an extension of the exposure period, a 4-week recovery period for 5 males/group, and sperm motility and concentration measurements. Rats were exposed by whole-body inhalation to cyclohexanol vapor at design exposure levels of 0, 50, 150 and 450 ppm (v/v), respectively. Male rats (15/group) were exposed 6 hours/day, 5 days/week for 16 weeks after which five males were selected for a 4-week recovery period. Female rats (15/group) for the reproductive component were exposed for 6 hours/day, 5 days/week for the first 10 weeks, and then 6 hours/day, 7 days/week for the next 6 weeks (during mating, gestation, and postpartum). After both males and females had been exposed to cyclohexanol for 10 weeks, the animals were cohabited, one repeat-dose male to one reproductive component female within the same treatment group, for up to 14 days. Females were allowed to deliver litters and maintain pups until Day 4 of lactation. Pups were euthanized and externally examined on Day 4 of lactation and the carcasses were discarded without further examination. Because of slight mortality seen at 450 ppm and concern about the effect of the additional stress during mating, the 450 ppm exposure level was lowered to 400 ppm at the beginning of the mating period (after 10 weeks exposure). Measurements on the animals included clinical signs, neurobehavioral observations, body weights and food consumption. Complete necropsies were performed on all adult animals and specific

organs and tissues were weighted and microscopically examined. Sperm motility and caudal epididymal concentration counts were performed for all males. Additionally, testicular sperm counts on the fresh tissue collected at necropsy were performed for the control and high exposure groups along with sperm morphology. Toward the end of the gestation period, females were examined twice daily for signs of parturition. Females were allowed to give birth (F_1). The duration of gestation was calculated and any difficulties occurring at parturition were recorded. The day on which all pups were delivered was designated as Day 0 of lactation. The litters were examined as soon as possible after delivery and parameters including litter size, number of stillborn and liveborn pups, and gross abnormalities were recorded. Pups were weighed, sexed, and examined for abnormalities on Days 0 and 4 of lactation. Any abnormal behavior observed in the pups was recorded daily. On Day 4 of lactation, the pups were euthanized, externally examined and discarded without further evaluation.

Results: The overall mean (±SD) exposure levels for the study were 50±9, 151±25, and 449±82 (1st 10 weeks) or 403±43 ppm (last 6 weeks) of cyclohexanol in air. Single males were found dead at 450 ppm on Days 37, 38 and 60 of the study. A single female was found dead on Day 31 and a single female was euthanized in extremis on Day 17. Microscopically, the cause of these deaths could not be determined but the deaths were considered to be test article-related since they all occurred at the high exposure level. No adverse dose-related effect was evident during the premating, gestation or lactation periods on clinical observations, body weight, body weight gain, food consumption, reproductive and fertility parameters, or litter size data. Pup sex ratios and pup survival to Lactation Day 4 were also unaffected by treatment. Two of the 11 pregnancies (18.2%) in the 450/400 ppm group resulted in no viable pups at parturition. Lower mean pup weights (10-12%) were also seen at birth and at Postnatal Day 4 in the high exposure group. Relative to microscopic examination of organ and tissues from adult males and females, no adverse histopathogy was seen at the end of the study (16 weeks). High-dose male rats showed a reduction in testicular sperm counts at completion of the exposure period (16 weeks).
However, these reduced counts at the high level were not considered to be toxicologically relevant because they were within the range of recent historical controls for this particular rat strain and because recovery high-level rats four weeks postexposure had sperm counts similar to concurrent controls. Thus, the NOEL for reproductive performance is 150 ppm, based on a few pregnancies with no viable fetuses and reduced F_1 pup weights at 450 ppm..

Conclusions: In the reproductive component of this modified OECD 422 Study, the only compound-related effects seen occurred at the 450/400 ppm level and included a slight increased incidence of pregnancies with no viable pups at birth and lower F, pup weights. Therefore, the 150 ppm cyclohexanol exposure level is considered the NOEL for reproductive performance.

Data Quality (Klimisch Code): **[2]** Valid with restrictions
Screening reproduction study

Reference: Newton, P.E. (MPI Research, Inc.). An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in Rats with Cyclohexanol, Report No. 683-004, 2005.

(B.) Additional Studies by Other Routes

Remarks: In a study by Tyagi et al. (1979), 20 adult male gerbils and 20 male rats were subcutaneously injected with 15 mg cyclohexanol/kg/day for a period of 21 and 37 days, respectively. A significant reduction in the weights of the testes, epididymides, seminal vesicles and ventral prostate was detected. In addition, the authors indicated, based on their histological evaluation, that spermatogenesis in both species was arrested. Recovery was not investigated. In another study (Dixit et al,. 1980), groups of 15 male rabbits received 25 mg cyclohexanol/kg/day by gavage for a period of 40 days. One group was allowed a 70-day recovery period following cessation of cyclohexanol administration. Similar to the preceding gerbil and rat findings, a significant reduction in the weights of the testis and epididymides was observed. Additionally, marked degenerative changes were noted upon microscopic examination of the testes. The changes were consistent with those previously described for the gerbil and the rat. Normal spermatogenesis was seen after 70 days following cessation of cyclohexanol treatment. The organ weights were also comparable to the controls. In a third study (Lake et al. 1982), male rates were given 455 mg cyclohexanol/kg/day by gastric intubation for 7 days. Cyclohexanol increased liver size and stimulated certain parameters of hepatic xenobiotic metabolism in the rat but had no effect on testis weight.

Reliability: Inadequate Information (No study meets HPV requirements)

References:

- Dixit, V.P. et al (1980). Reversible Chemical Sterilization: Effects of Cyclohexanol Administration on the Testes and Epididmides of the Rabbits. Indian J. Physiol. Pharmacol. 24: 278-286.
- Lake, B.G. et al. (1982). Studies on the Effects of Orally Administered Dicyclohexyl Phthalate in the Rat. Acta Pharmacol. Toxicol. 51: 217-226.
- Tyagi, A. et al. (1979). Antispermatogenic Activity of Cyclohexanol in the Gerbil and House Rat. Indian Journal of Experimental Biology 17: 1305-1307.

5.8 DEVELOPMENTAL TOXICITY

Type: An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in Rats: Developmental Toxicity Component.

Species/strain: Sprague-Dawley [Crl: CD®(SD)IGS BR] Female Rats (15/exposure group)

Route of Administration: Inhalation

Exposure period: 10 weeks prior to mating and then for up to 6 more weeks including mating, gestation and postpartum (except from Gestation Day 21 to Postpartum Day 3).

Frequency of Treatment: 6 hours/day, 5 days/week for 10 weeks, then for 7 days/week for up to 6 weeks (during mating, gestation and postpartum)

Exposure Levels: 0, 50, 150 and 450/400 ppm (v/v); after 10 weeks exposure, the 450 ppm level was reduced to 400 ppm due to slight mortality and the perceived additional stress of mating.

Control group: Yes (air alone)

Method: As part of a modified OECD Guideline 422 Study, a developmental toxicity screening study was conducted. The only modification to the original OECD 422 protocol was an expansion of the exposure period to 10 weeks prior to mating. After both males (15/exposure level) (from the Repeat Dose Component of the OECD 422 Study) and females (15/exposure level; separate group from the females in the Repeat Dose Component of the OECD 422 Study) had been exposed to cyclohexanol (0, 50, 150 and 450/400 ppm) for 10 weeks, the animals were cohabited, one repeat-dose male to one reproductive component female within the same treatment group, for up to 14 days. Females were then allowed to deliver their litter naturally and maintain their pups until Day 4 of lactation. Pups were then euthanized and externally examined on Day 4 of lactation and the carcasses were discarded without further examination. Because of slight mortality seen at 450 ppm and concern about the effect of the additional stress of mating, the 450 ppm exposure level was lowered to 400 ppm at the beginning of the mating period (i.e., after 10 weeks exposure). Measurements on the female rats included clinical signs, neurobehavioral observations, body weights and food consumption. Microscopic examination of organs and tissues was conducted on reproductive females at the termination of the study. Toward the end of the gestation period, females were examined twice daily for signs of parturition. Females were allowed to give birth naturally. The duration of gestation was calculated and any difficulties occurring at parturition were recorded. The day on which all pups were delivered was designated as Day 0 of lactation. The litters were examined as soon as possible after delivery and parameters including litter size, number of stillborn and liveborn pups, and gross abnormalities of the pups were recorded. Pups were weighed, sexed, and examined for abnormalities on Days 0 and 4 of lactation. Any abnormal behavior observed in the pups was recorded daily. On Day 4 of lactation, the pups were euthanized, externally examined, and discarded without further evaluation.

Results: In the screening developmental toxicity component of this OECD 422 Study, the overall mean exposure levels were 50±9, 151±25, 449±82 (1st 10 weeks prior to mating) and 403±43 ppm (last 6 weeks of the study). The number of females that delivered litters in the 0, 50, 150 and 450/400 ppm groups were 14, 15, 15, and 10, respectively; each exposure group started with 15 female rats each. No adverse dose-related effect was evident during the premating, gestation or lactation periods relative to

clinical observations, body weight, body weight gain, food consumption, reproductive and fertility parameters, and litter size data. Pup sex ratio and pup survival to Lactation Day 4 were also unaffected by treatment. Two of the 11 pregnancies (18.2%) in the 450/400 ppm group resulted in no viable pups at parturition. Lower mean pup weights were also seen at birth and at Postnatal Day 4 in this high exposure group. No evidence of terata was evident from the clinical examination of the pups. Microscopic examination of female rat organs and tissues at study termination was also unremarkable. It is important to note that in the repeated dose portion of this OECD 422 study, a slight mortality increase and prostration immediately post-exposure also occurred at the high exposure level. The NOEL for developmental toxicity in this study was considered to be 150 ppm.

Conclusions: In the developmental toxicity component of this modified OECD 422 Study, female rats were exposed to cyclohexanol for 10 weeks prior to mating and then for up to 6 weeks thereafter – during mating, lactation and postpartum (except from Gestation Day 21 to Postpartum Day 3). The female rats were then mated to male rats that had been exposed to cyclohexanol for at least 10 weeks. Female rats showed no adverse clinical signs and the pups showed no evidence of terata at any exposure level. At the highest exposure level (450/400 ppm), the only treatment-related effects were a slight increased incidence of pregnancies with no viable fetuses and lower pup weights. Therefore, the NOEL for developmental toxicity was considered to be 150 ppm.

Data Quality (Klimisch Code): **[2]** valid with restrictions
Screening Study
Longer premating exposure (10 weeks)

Reference: Newton, P.E. (MPI Research, Inc.). An Inhalation Combined Repeat Dose Toxicity Study and Reproduction/Developmental Toxicity Screening Study (OECD 422) in Rats with Cyclohexanol. Report No. 683-004, 2005.

5.11 EXPERIENCE WITH HUMAN EXPOSURE (WORKPLACE)

Remarks: The five US producers of cyclohexanone/cyclohexanol have, on various occasions between 1994-2000, taken area and/or personal samples for determination of possible exposure to cyclohexanol. Information has been submitted to IHF as Agent for the Consortium. To preserve the confidentiality of individual Company data, the details may be summarized as follows:

1. Samples were collected on either charcoal tubes or charcoal badges and analysed by gas chromatography using flame ionisation detection methodology.
2. The lower limits of detection varied from about 0.01 ppm for the longer-term samples (8 hours) to 0.4 ppm for short-term samples (15 minutes to one hour).
3. Area samples (n>200) and personal samples (n=200) ranged from averages of 0.01-3.5 ppm for longer sampling intervals and averages of 0.4-29 ppm for short sampling intervals.

4. Area samples tended to be of long duration with results only slightly above the appropriate detection limits for the majority of samples.
5. None of the samples taken suggested the probability of exposure in excess of the current OSHA PEL/ACGIH TLV® of 50 ppm.

Reference: Industrial Health Foundation, Pittsburgh, PA, June 15, 2001

6.0 REFERENCES